abcam

Product datasheet

Anti-BRG1 antibody [EPNCIR111A] ab110641





重组 RabMAb

★★★★★ 9 Abreviews 164 References 19 图像

概述

产品名称 Anti-BRG1抗体[EPNCIR111A]

描述 兔单克隆抗体[EPNCIR111A] to BRG1

宿主 Rabbit

经测试应用 适用于: Flow Cyt (Intra), ChIC/CUT&RUN-seq, WB, IP, IHC-P, ICC/IF

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 WB: Hap1, HEK-293T, K562, HeLa, MOLT4, NIH3T3, RAW 264.7, C6 and PC12 cell lysates;

> Human kidney and testis tissue lysates. IHC-P: Human testis, colon, cervical carcinoma and kidney tissue, rat liver tissue and mouse kidney tissue. ICC/IF: HeLa cells, Raji and SMARCA4-

HAP1 cells. Flow Cyt (intra): HeLa and HAP1 cells. ChlC/CUT&RUN-Seq: HeLa cells.

常规说明 This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

This antibody was developed as part of a collaboration between Epitomics, the National Cancer Institute's Center for Cancer Research and the lab of Gordon Hager. View antibodies from NCI

Center for Cancer Research Collaboration.

性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Stable for 12 months at -20°C.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

纯**度** Protein A purified

克隆 单克隆

克隆编号 EPNCIR111A

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab110641于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/200.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg
WB	★★★★★ (5)	1/10000 - 1/50000. Predicted molecular weight: 185 kDa.
IP	★★★★ (1)	1/10 - 1/100.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF	★★★★★ (2)	1/500.

靶标

功能

Transcriptional coactivator cooperating with nuclear hormone receptors to potentiate transcriptional activation. Component of the CREST-BRG1 complex, a multiprotein complex that regulates promoter activation by orchestrating a calcium-dependent release of a repressor complex and a recruitment of an activator complex. In resting neurons, transcription of the c-FOS promoter is inhibited by BRG1-dependent recruitment of a phospho-RB1-HDAC repressor complex. Upon calcium influx, RB1 is dephosphorylated by calcineurin, which leads to release of the repressor complex. At the same time, there is increased recruitment of CREBBP to the promoter by a CREST-dependent mechanism, which leads to transcriptional activation. The CREST-BRG1 complex also binds to the NR2B promoter, and activity-dependent induction of NR2B expression involves a release of HDAC1 and recruitment of CREBBP. Belongs to the neural progenitors-specific chromatin remodeling complex (npBAF complex) and the neuronspecific chromatin remodeling complex (nBAF complex). During neural development a switch from a stem/progenitor to a post-mitotic chromatin remodeling mechanism occurs as neurons exit the cell cycle and become committed to their adult state. The transition from proliferating neural stem/progenitor cells to post-mitotic neurons requires a switch in subunit composition of the npBAF and nBAF complexes. As neural progenitors exit mitosis and differentiate into neurons, npBAF complexes which contain ACTL6A/BAF53A and PHF10/BAF45A, are exchanged for homologous alternative ACTL6B/BAF53B and DPF1/BAF45B or DPF3/BAF45C subunits in neuron-specific complexes (nBAF). The npBAF complex is essential for the selfrenewal/proliferative capacity of the multipotent neural stem cells. The nBAF complex along with CREST plays a role regulating the activity of genes essential for dendrite growth.

SMARCA4/BAF190A may promote neural stem cell self-renewal/proliferation by enhancing Notch-dependent proliferative signals, while concurrently making the neural stem cell insensitive to SHH-dependent differentiating cues (By similarity). Also involved in vitamin D-coupled transcription regulation via its association with the WINAC complex, a chromatin-remodeling complex recruited by vitamin D receptor (VDR), which is required for the ligand-bound VDR-mediated transrepression of the CYP27B1 gene. Acts as a corepressor of ZEB1 to regulate E-cadherin transcription and is required for induction of epithelial-mesenchymal transition (EMT) by ZEB1.

组织特异性

Colocalizes with ZEB1 in E-cadherin-negative cells from established lines, and stroma of normal colon as well as in de-differentiated epithelial cells at the invasion front of colorectal carcinomas (at protein level).

疾病相关

Defects in SMARCA4 are the cause of rhabdoid tumor predisposition syndrome type 2 (RTPS2) [MIM:613325]. RTPS2 is a familial cancer syndrome predisposing to renal or extrarenal malignant rhabdoid tumors and to a variety of tumors of the central nervous system, including choroid plexus carcinoma, medulloblastoma, and central primitive neuroectodermal tumors. Rhabdoid tumors are the most aggressive and lethal malignancies occurring in early childhood.

序列相似性

Belongs to the SNF2/RAD54 helicase family.

Contains 1 bromo domain.

Contains 1 helicase ATP-binding domain. Contains 1 helicase C-terminal domain.

Contains 1 HSA domain.

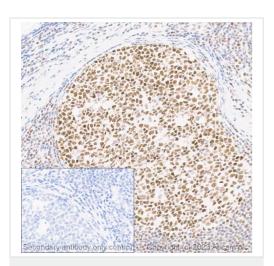
翻译后修饰

Phosphorylated upon DNA damage, probably by ATM or ATR.

细胞定位

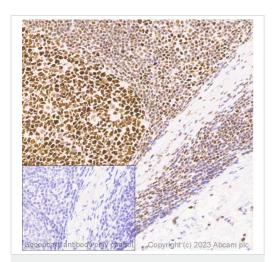
Nucleus.

图片



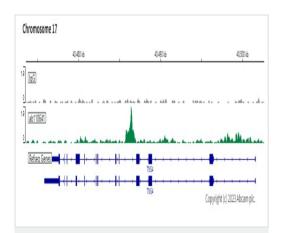
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BRG1 antibody
[EPNCIR111A] (ab110641)

Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil labelling BRG1 with ab110641 at a concentration of 0.1 µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32 mins at 100°C with ULTRA cell conditioning solution (CC1, pH 8.5). ab110641 anti-BRG1 antibody [EPNCIR111A] was incubated at 37°C for 16 mins. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BRG1 antibody
[EPNCIR111A] (ab110641)

Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil labelling BRG1 with ab110641 at a concentration of 0.1 µg/ml. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with a Bond™ Polymer Refine Detection kit. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution 2) for 20 mins. ab110641 anti-BRG1 antibody [EPNCIR111A] was incubated for 30 mins at room temperature. Sections were counterstained with Hematoxylin. Image inset shows absence of staining in secondary antibody only control.

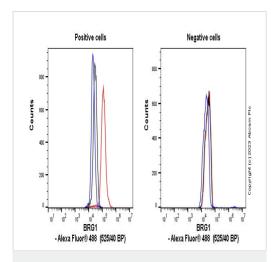


ChIC/CUT&RUN sequencing - Anti-BRG1 antibody [EPNCIR111A] (ab110641)

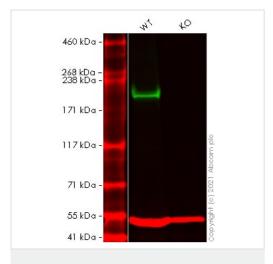
ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 5µg of ab110641 [EPNCIR111A]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control ab172730 is also shown.

Additional screenshots of mapped reads can be downloaded <u>here</u>.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Flow Cytometry (Intracellular) - Anti-BRG1 antibody [EPNCIR111A] (ab110641)



Western blot - Anti-BRG1 antibody [EPNCIR111A] (ab110641)

Flow cytometry overlay histogram showing left wild-type Hap1 positive cells and right negative SMARCA4 knockout Hap1 stained with <u>ab95363</u> (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab110641) (1x 10^6 in 100μ I at 0.008 μ g/mI (1/266250)) for 30min at 22° C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (black line) was used at the same concentration and conditions as the primary antibody. Unlabelled sample was also used as a control (blue line).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

All lanes : Anti-BRG1 antibody [EPNCIR111A] (ab110641) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: SMARCA4 knockout HEK-293T cell lysate

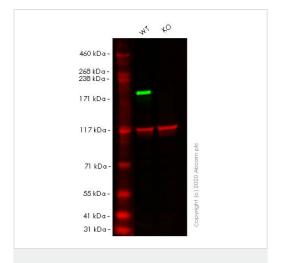
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 185 kDa **Observed band size:** 185 kDa

False colour image of Western blot: Anti-BRG1 antibody
[EPNCIR111A] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab110641 was shown to bind specifically to BRG1. A band was observed at 185 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in SMARCA4 knockout cell line ab255432 (knockout cell lysate ab263853). To generate this image, wild-type and SMARCA4 knockout HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in

TBS-0.1 % Tween[®] 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit lgG H&L (IRDye[®] 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye[®] 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Anti-BRG1 antibody [EPNCIR111A] (ab110641)

All lanes : Anti-BRG1 antibody [EPNCIR111A] (ab110641) at 1/10000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: SMARCA4 knockout HEK-293T cell lysate

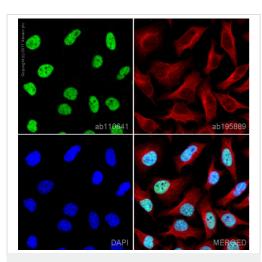
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 185 kDa **Observed band size:** 185 kDa

Lanes 1-2: Merged signal (red and green). Green - ab110641 observed at 185 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) observed at 50 kDa.

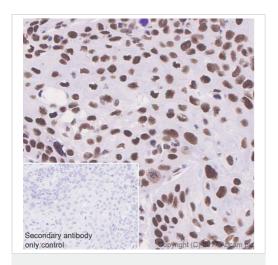
ab110641 was shown to react with SMARCA4 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line ab255432 (knockout cell lysate ab263853) was used. Wild-type HEK-293T and SMARCA4 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab110641 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-BRG1 antibody [EPNCIR111A] (ab110641)

ab110641 staining BRG1 in HeLa cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab110641 at 1/500 dilution and ab195889 (Mouse monoclonal [DM1A] to alpha Tubulin - Microtubule Marker (Alexa Fluor® 594)) at 1/250 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with ab150081 (Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488)) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

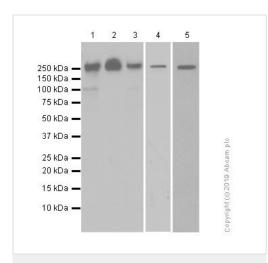
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BRG1 antibody
[EPNCIR111A] (ab110641)

Immunohistochemical analysis of paraffin-embedded Human cervical carcinoma tissue labeling BRG1 with ab110641, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining on human cervical carcinoma. Counterstained with Hematoxylin. Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP).



Western blot - Anti-BRG1 antibody [EPNCIR111A] (ab110641)

All lanes : Anti-BRG1 antibody [EPNCIR111A] (ab110641) at 1/10000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lane 2: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates
Lane 3: RAW 264.7(Mouse Abelson murine leukemia virus-

induced tumor macrophage)whole cell lysates

Lane 4: C6 (Rat glial tumor glial cell) whole cell lysates

Lane 5: K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysates

Lysates/proteins at 20 µg per lane.

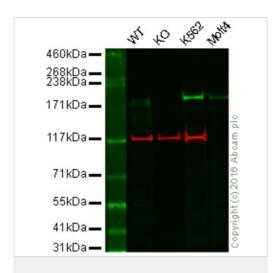
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 185 kDa Observed band size: 185 kDa

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: Lane 1 to 4: 5 seconds; Lane 5: 15 seconds



Western blot - Anti-BRG1 antibody [EPNCIR111A] (ab110641)

All lanes : Anti-BRG1 antibody [EPNCIR111A] (ab110641) at 1/10000 dilution

Lane 1: Wild-type HAP1 cell lysate

Lane 2: BRG1 knockout HAP1 cell lysate

Lane 3 : K562 cell lysate

Lane 4 : Molt-4 cell lysate

Lysates/proteins at 20 µg per lane.

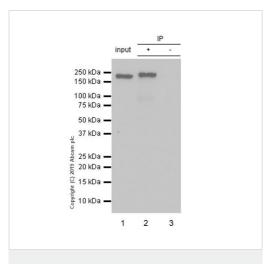
Predicted band size: 185 kDa

Additional bands at: 185 kDa. We are unsure as to the identity of

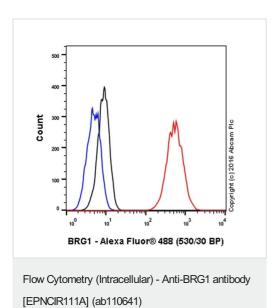
these extra bands.

Lanes 1 - 4: Merged signal (red and green). Green - ab110641 observed at 185 kDa. Red - loading control, **ab18058**, observed at 124 kDa.

ab110641 was shown to specifically react with BRG1 in wild-type HAP1 cells. No band was observed when BRG1 knockout samples were used. Wild-type and BRG1 knockout samples were subjected to SDS-PAGE, ab110641 and ab18058 (loading control to Vinculin) were both diluted 1/10,000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10,000 dilution for 1hr at room temperature before imaging.



Immunoprecipitation - Anti-BRG1 antibody [EPNCIR111A] (ab110641)



BRG1 was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 μ g with Ab110641 at 1:20 dilution (0.3 μ g in 0.35mg lysates). Western blot was performed on the immunoprecipitate using Ab110641 1:1000 dilution (0.12 μ g/ml). VeriBlot for IP Detection Reagent (HRP) (ab131366) was used as the secondary antibody at 1:1000 dilution.

Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg

Lane 2: Ab110641 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal lgG ($\underline{ab172730}$) instead of ab110641 in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

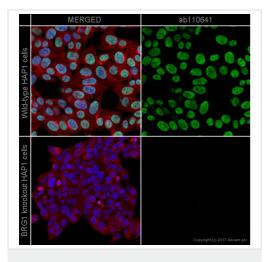
Exposure time: 1 second

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling BRG1 with purified ab110641 at 1/200 dilution (10µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit lgG (Alexa Fluorr $^{\textcircled{\tiny{\$}}}$ 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal lgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Different batches of ab110641 were tested on HeLa (Human cervix adenocarcinoma epithelial cell) lysate at 0.5 μ g/ml. 15 μ g of lysate was loaded in each lane. Bands observed at 185 kDa.

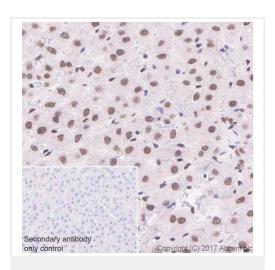
Western blot - Anti-BRG1 antibody [EPNCIR111A] (ab110641)



Immunocytochemistry/ Immunofluorescence - Anti-BRG1 antibody [EPNCIR111A] (ab110641)

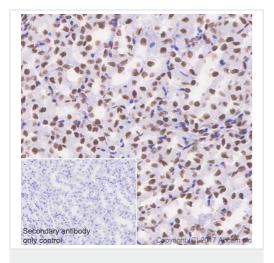
ab110641 staining BRG1 in wild-type HAP1 cells (top panel) and BRG1 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab110641 at 1/500 dilution and ab195889 at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 μ g/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BRG1 antibody
[EPNCIR111A] (ab110641)

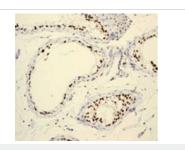
Immunohistochemical analysis of paraffin-embedded Rat liver tissue labeling BRG1 with ab110641, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining on rat liver. Counterstained with Hematoxylin. Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BRG1 antibody
[EPNCIR111A] (ab110641)

Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labeling BRG1 with ab110641, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining on mouse kidney. Counterstained with Hematoxylin. Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BRG1 antibody
[EPNCIR111A] (ab110641)

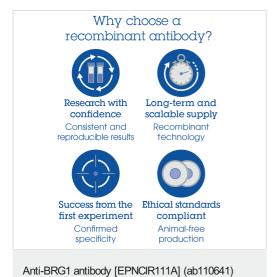
Immunohistochemical analysis of paraffin-embedded human testis tissue staining BRG1 with ab110641 at 1/100 dilution. Heat mediated antigen retrieval was performed with citrate buffer (pH 6).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BRG1 antibody

[EPNCIR111A] (ab110641)

Immunohistochemical analysis of paraffin-embedded human kidney tissue staining BRG1 with ab110641 at 1/100 dilution. Heat mediated antigen retrieval was performed with citrate buffer (pH 6).



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